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## NOTES ON BULBIFEROUS FUNGI WITH A KEY TO DESCRIBED SPECIES

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(WITH PLATES XXI–XXIII AND SIX FIGURES)

### Introduction

As has been shown in a former article (**6**), the term bulbil, as applied to fungi, refers to reproductive bodies of more or less definite form, composed of a compact mass of homogeneous or heterogeneous cells which may be few or many in number, but which are usually developed from primordia of more than one cell. This mode of reproduction is common among certain fungi and constitutes the only known means among others. Many of these structures superficially resemble the "spore-balls" of *Urocystis* or *Tuburcinia* among the smuts, but differ from them in their manner of germination. In general appearance and mode of development the bulbils of *Papulospora spinulosa* Hotson might readily be taken for "spore-balls" of *Urocystis*, but, on germination, promycelia bearing sporidia such as are produced by the smuts are not formed, nor is the production of these structures ever associated with the germination of bulbils. Other bulbils resemble compound spores of the *Stemphilium* type, but the latter are the result of successive divisions of single cells, while bulbils are derived from a group of initial cells to which new ones are added by a process of gemmation rather than formed by the septation of a single cell. Although this is true in general, in compound spores like those of

*Stephanoma strigosum* Wallr. the superficial cells are produced in a manner similar to those of certain bulbils. Mature bulbils may also resemble sclerotia. The latter, however, may be regarded as the result of the irregular massing together of vegetative filaments, the individual cells of which do not partake of the nature of spores either in appearance or structure, while in the bulbil those cells that are filled with protoplasm usually act independently of each other, in this respect resembling spores. There are a number of sclerotia of the simpler type, such as are produced by *Penicillium italicum* and its allies, which are small and more or less regular in form and outline, somewhat resembling bulbils in appearance. The mode of development of these sclerotia, however, consists in the irregular massing together of the vegetative filaments, as has just been mentioned.

Before 1912 the literature relating to bulbils dealt with less than a dozen described forms. Most of these were referred either to the form genus *Papulospora* or to *Helicosporangium*. Owing to the fact that the limitations of these two genera were not clearly defined, it was thought wise to redescribe the genus *Papulospora* (6), and to group all those fungi that produced bulbils, but whose perfect condition had not been obtained, into this form genus. The literature on this subject has been carefully reviewed in the article already mentioned. This article shows clearly that these fungi do not belong to any one of the natural orders, nor do they in any sense form a group by themselves, but occur without regularity as imperfect forms among the main groups of higher fungi. The forms, associated with bulbiferous conditions mentioned in that article, include among the Discomycetes a new species of *Cubonia*, among the Hypocreales 3 species of *Melanospora;* among the Basidiomycetes at least 4 types; while 9 species of *Papulospora* as yet unconnected with a perfect form are added to those already known. Among the latter, also, *Papulospora candida* Sacc. was found to be definitely connected with a second and well marked imperfect form, namely *Verticillium agaricinum* (Link) Corda var. *clavisedum* Sacc.

In 1914 NEGER (9) referred to a bulbiferous condition in connection with the life history of *Melanospora marchica* Lindau.

According to his account, the bulbils resemble the compound spores of *Urocystis* among the Ustilaginales. The color of these *Urocystis*-like spores is reddish to chocolate brown, their form more or less spherical, the cortex being a layer of empty, colorless cells. The size of the spore balls, however, is not given. They apparently resemble the bulbils of *Melanospora papillata* Hotson, but the perithecia of the two species are different. In *M. marchica* the perithecium has no papilla, the setae arising from the flush surface of the wall. The perithecium of *M. papillata*, on the other hand, has a distinct and often quite prominent papilla, the terminal setae being produced at its tip. The bulbils of *M. marchica* also resemble those of *Papulospora coprophila* (Zukal) Hotson, but vary somewhat in their mode of development. Apparently NEGER had not seen the writer's article dealing with bulbils (6) or that of BAINIER (1), and therefore makes no comparisons.

Recently DODGE (3, 4) has reported a species of *Papulospora* closely associated with *Ascobolus magnificus* Dodge. He is of the opinion that this is either a parasite on or an asexual spore form of the *Ascobolus*. These bulbils are light brown, with a layer of empty cells forming the margin. A description of this fungus is given in the present article under the name of *Papulospora magnifica*.

It has been shown in a recent article by MELHUS, ROSENBAUM, and SCHULTZ (8) that a species of *Papulospora* producing bulbils is frequently associated with the powdery scab of potatoes (*Spongospora subterranea* [Wallr.] Johnson). These investigators have isolated *P. coprophila* (Zukal) Hotson from tubers infected with powdery scab. This organism has been shown by inoculation experiments to be entirely saprophytic and in no way responsible for the disease. They believe, however, that the presence of bulbils of a species of *Papulospora* associated with *Spongospora* in the same sorus has probably been largely responsible for the confusion found in the writings of earlier investigators who observed numerous fungous threads in the sori, and in some instances spore balls that were quite different from those of *Spongospora*.

The cultural methods used in the study of the forms under consideration were similar to those described in a former paper (6).

The substrata were put in moist chambers, and as the bulbils appeared they were picked out with sterilized dissecting needles and transferred to tubes containing nutrient material.

## Description of species

Bulbils are in all cases to be regarded as imperfect conditions of higher fungi. As has already been indicated, some have been definitely connected with perfect conditions belonging to widely separated genera of both Ascomycetes and Basidiomycetes. Those, however, that are to be considered in the present article have thus far baffled every effort to induce them to produce any perfect form, even after 7 or 8 years of cultural study. Two of these are doubtless Basidiomycetes, since their mycelia possess clamp connections, while another shows some evidence that it belongs to the Pyrenomycetes. It is the aim of the present article to contribute further information regarding the occurrence, morphology, and development of bulbils, and also to bring together the described species in the form of a key to the genus *Papulospora*.

**Papulospora pallidula**, n.sp. (figs. 1–16; text fig. 2).—Mycelium white, procumbent, scanty on most media; bulbils colorless, becoming pale yellow when old, somewhat spherical, 70–100 $\mu$ in diameter, sometimes elongated to 140 $\mu$; primordium of two kinds, one a short lateral branch which divides dichotomously of 3 or 4 orders, occasionally more, and the other a group of intercalary cells. No other means of reproduction at present known.

On gross cultures of dog dung from Guatemala and Claremont, California; also on rabbit dung from Ontario.

The substrata were put into moist chambers and, when the bulbils appeared, transfers were made, eventually producing pure cultures. This fungus has been kept growing for more than 7 years on many different kinds of media, such as bran, prune, potato, cornmeal agar, etc. Thus far, however, all efforts to induce it to produce any other fructification than bulbils have failed. The bulbils are readily distinguished by their pale color. On a clear substratum they are almost colorless, while on horse dung or other dark media they become slightly yellowish or cream colored. As

is true with many other fungi, the abundance of the mycelium depends largely upon the kind of substratum. On potato or goat dung agar it develops very sparingly, often becoming quite difficult to detect even with a good lens, while on bran or cornmeal agar it becomes more conspicuous, growing evenly over the whole surface of the culture and on the sides of the tubes, but never becoming very flocculent. On appropriate media such as horse dung, bran, or cornmeal agar, the mycelium forms a thick felted layer over the substratum. Most of the hyphae are small, about 3–5 $\mu$ in diameter, but some of the older ones become as large as 10 $\mu$, with prominent cross walls. They are frequently packed with large oil globules (fig. 1). Here and there in the hyphae swollen cells appear that are full of food material. These are oval at first, but eventually become almost spherical.

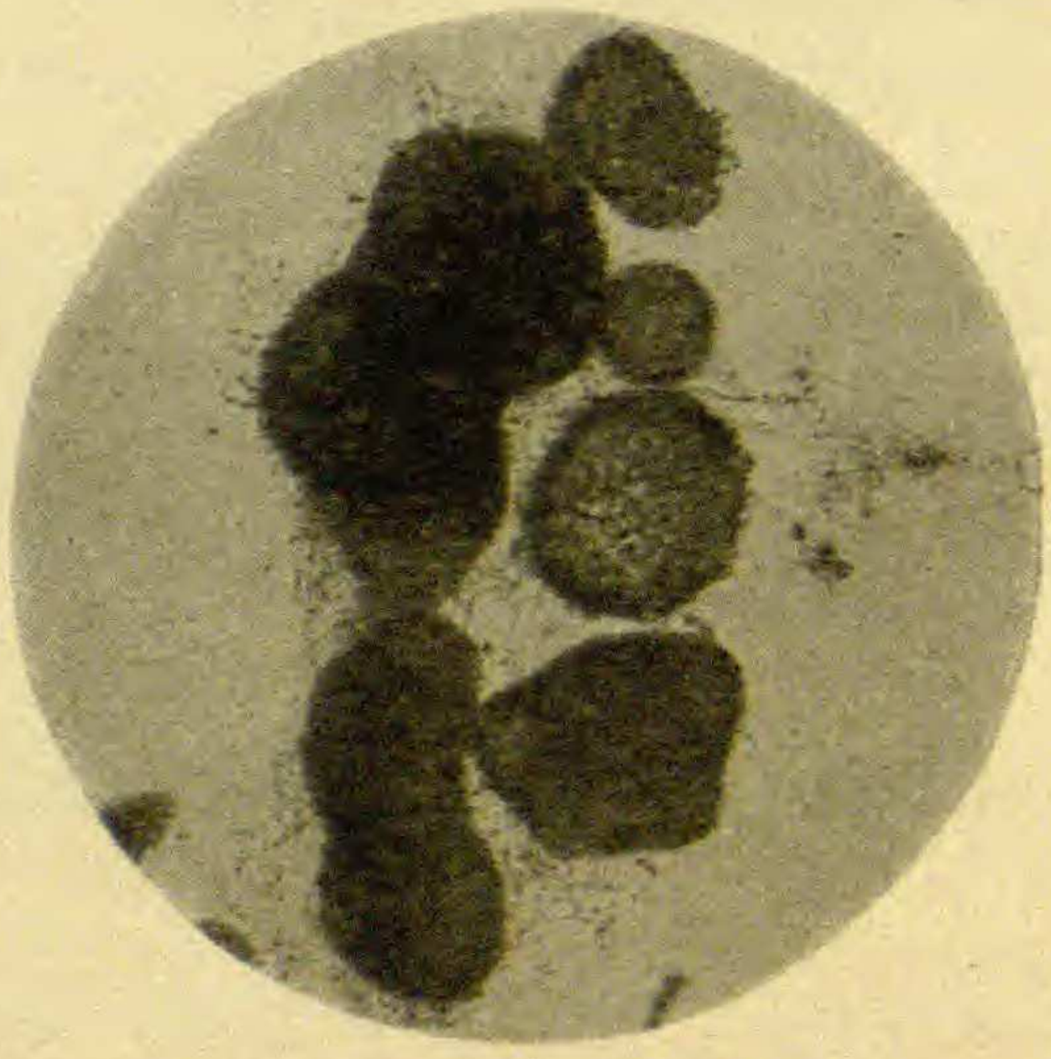


FIG. 1.—Group of bulbils of *P. byssina*, showing variations in size.

DEVELOPMENT OF BULBIL.—A short lateral branch divides dichotomously, producing dichotomies of the second, third, or sometimes of the fourth order (figs. 2–6). These branches divide into short cells which enlarge, eventually forming the central ones of the bulbil. As these cells grow they become more compact, and from them by a process of budding others are formed which increase in size, becoming closely and compactly pressed against their neighbors (figs. 7–9). This mode of development usually produces mature bulbils that are more or less spherical in form and measure 70–100 $\mu$ in diameter (fig. 13).

A second mode of development of the bulbil is sometimes observed. Intercalary cells become swollen, having absorbed a large quantity of food material. From these large cells others are produced by a process of gemmation, and these in turn bud off others and so on (figs. 10–12). Eventually a bulbil that is very

pale in color is formed, with several large cells in the center which are conspicuously filled with oil globules. These bulbils are usually more or less spherical, but not infrequently become elongated, as shown in text fig. 2, which shows a group of bulbils, the longest of which measures 78 by 140 $\mu$ and is probably the result of the fusing of two immature forms. These bulbils germinate readily in water. Fig. 13 shows a germinating bulbil 75 by 67.5 $\mu$ in diameter after 24 hours in a Van Tieghem cell. The young hyphae, which have a large number of oil globules, are usually produced from the larger cells, but any cell is capable of germination. Occasionally as the bulbils grow older the cells composing them adhere less firmly together, becoming more and more like independent spores (fig. 14). It requires only a slight pressure to force them apart. This resembles the condition found in connection with the development of the bulbil of *Papulospora polyspora* Hotson (6), which in turn suggests a similar condition found in *Aegerita webberi* Fawcett (5). Fig. 14 shows a few of these loosely connected cells germinating, which seem to act independently, like spores.

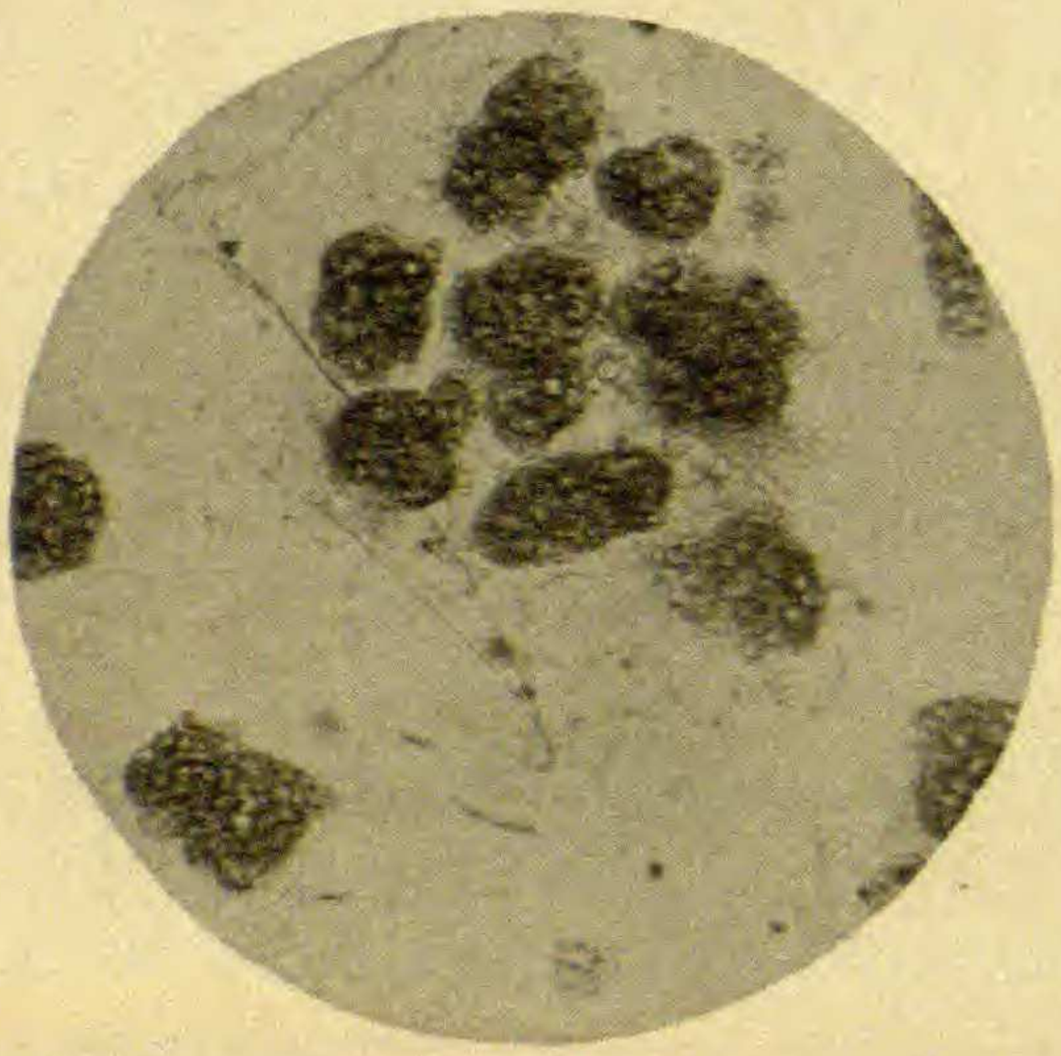


FIG. 2.—Group of bulbils of *P. pallidula*.

It is probable that one of the primordia just described is that of the perfect stage, but for some reason it fails to develop further. This view is strengthened by the fact that on several occasions perithecium-like structures over a millimeter in diameter have been found, as if an effort were being made by the fungus to produce the perfect stage. Thus far, however, none of these have been induced to develop sufficiently to produce spores.

**Papulospora byssina,** n.sp. (figs. 17–24; text fig. 1).—Mycelium white, procumbent, scanty on most media; bulbils light straw or cream colored, becoming brownish with age, more or less spherical

in form, 100–250 $\mu$, occasionally elongated to 350 $\mu$ in diameter, produced in fluffy aerial clusters; primordium one or more short lateral branches twining spirally about the main branch. No other means of reproduction at present known.

On horse dung, Kittery, Maine; Seattle, Washington; St. Louis, Missouri.

The original material from which pure cultures of this fungus were obtained was found on a horse dung compost at Kittery, Maine, by Dr. THAXTER. It has since been found by the writer on similar material in the vicinity of Seattle; also on material sent from St. Louis, Missouri, by S. M. ZELLER. In the last instance the bulbils apparently were produced after the horse dung compost had been used as a fertilizer on mushroom beds. This fungus has never been found on any other substratum than horse dung. It has been grown on different media in pure cultures for 6 years without inducing it to produce any other fructification than bulbils.

The mycelium is white, 3–5 $\mu$ in diameter. It is usually procumbent, but when cultures are left in such a position that the hyphae can grow straight downward they grow out into the air, producing long streamers or festoons which attach themselves to the opposite side of the test tube.

The bulbils of *P. byssina* resemble those of *Grandinia crustosa*, but the two species can easily be distinguished by the prominent clamp connections in the mycelium of the latter. Even the general appearance of the mycelium in cultures is sufficient to distinguish them, *Grandinia* producing characteristic "white, fibrous, ropelike strands of hyphae which radiate conspicuously in all directions from the point of inoculation." This phenomenon is entirely absent in *P. byssina*. The cells composing the bulbils are homogeneous throughout. In this respect they resemble those of *P. sporotricoides* and *P. cinerea*, but in the former species the bulbils are small, 20–36 $\mu$ in diameter, and chocolate brown, while in the latter they are about the same size but steel gray. In *P. byssina*, however, the bulbils are large, 100–250 $\mu$ in diameter, and straw color.

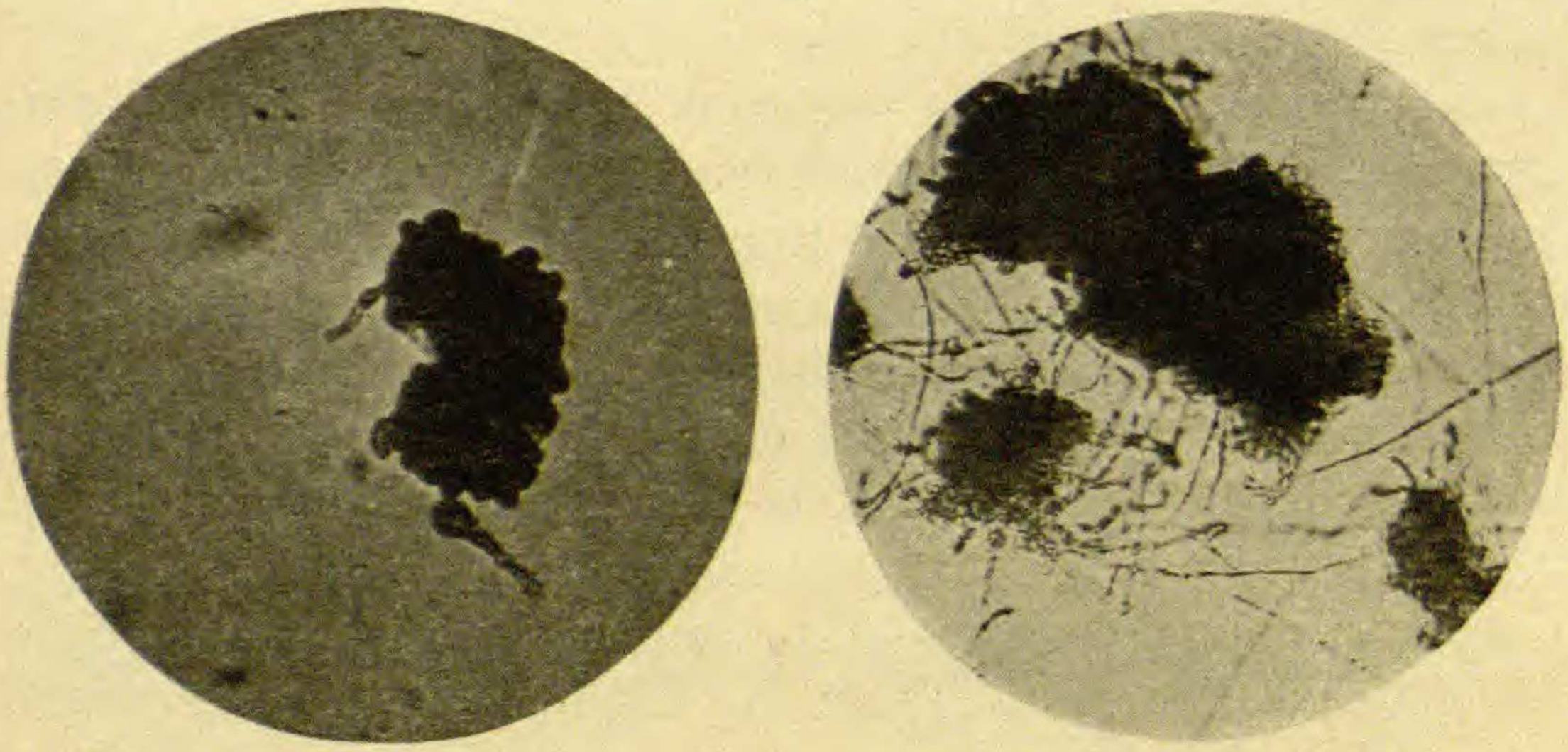
DEVELOPMENT OF BULBIL.—In most cases the primordium of the bulbil consists of a short lateral branch which may or may not divide dichotomously. This branch coils around the main hypha

(fig. 17), which may send out other lateral branches near the first (fig. 19). This branch may also take part in the formation of the bulbil. The cells of these various branches increase in size and become well supplied with food material. From them short lateral branches are produced which either intertwine among each other, or if very short assume the form of new cells as if produced by a process of budding or gemmation. Fig. 20 shows the primordium of a bulbil in which the secondary branches are being produced. These will eventually intertwine with each other in a more or less wormlike fashion, as shown in fig. 21, an immature bulbil 44 $\mu$ in diameter. The first lateral branches that twine around the primary filament may become localized, in which case the mature bulbils will be somewhat spherical, as shown in fig. 22, which represents a bulbil 110 $\mu$ in diameter. More often, however, the spherical bulbils are produced in a slightly different way. Not infrequently a terminal branch coils up and winds back on itself, or it may divide dichotomously, both branches thus formed twining back on the main filament (fig. 18). A primordium of this sort develops in the same way as the one already described, by the intertwining of lateral branches. The mature bulbil, however, tends to be more spherical than that in which a lateral branch twines about the primary filament. Occasionally several bulbils may be produced from the same filaments, as is indicated in fig. 24, which shows the beginnings of 3 bulbils at *a*, *b*, and *c* respectively. At *a* the secondary branches are beginning to be formed in a manner similar to that shown in fig. 20. It is possible that *a* and *b* will merge into one, forming an elongated and more or less irregular bulbil (text fig. 1). Owing to the variation in the mode of development, a great diversity of form is produced. Text fig. 1 represents a group of bulbils showing this wide variation of form. The exact dimensions of these bulbils vary from 112.5 to 338 $\mu$, but occasionally even a greater difference than this is observed.

To test the germinating power of these fruiting bodies, hanging drops were made in Van Tieghem cells. It was found that in 24 hours many of them had begun to germinate, and in 48 hours numerous hyphae were developed. Fig. 23 represents a portion of a germinating bulbil after 48 hours.

**Papulospora aurantiaca,** n.sp. (figs. 25–38; text figs. 3, 4).—Mycelium white at first, becoming yellowish with age, procumbent, scanty on most media, densely filled with oil globules, clamp connections sparingly produced; bulbils pale yellow, becoming orange, nearly spherical, frequently aggregated, 100–250 $\mu$ in diameter; primordium a spiral of one or two turns. No other mode of reproduction at present known.

On bark collected by Dr. THAXTER near Port of Spain, Trinidad, W.I.



FIGS. 3, 4.—*P. aurantiaca:* fig. 3, mature bulbil; fig. 4, several germinating bulbils.

The mycelium of *Papulospora aurantiaca* is somewhat inconspicuous, the hyphae being small, usually about 2.5–3.5 $\mu$ in diameter, and scanty. On certain media, like cornmeal or bran agar, it becomes more marked but never profuse on any media tried. These included such nutrient material as potato, sugar, bran, cornmeal, prune juice, horse dung, various kind of wood, etc. The hyphae contain large numbers of oil globules which vary considerably in size. When the filaments are crushed these float out into the water, a number frequently fusing together and sometimes forming large spherical globules 17.5 $\mu$ or more in diameter.

Many and varied experiments have been made in the hope of causing the fungus to produce its perfect form, but thus far all efforts have failed. That it is a Basidiomycete is readily seen by the presence of clamp connections in the mycelium. These are

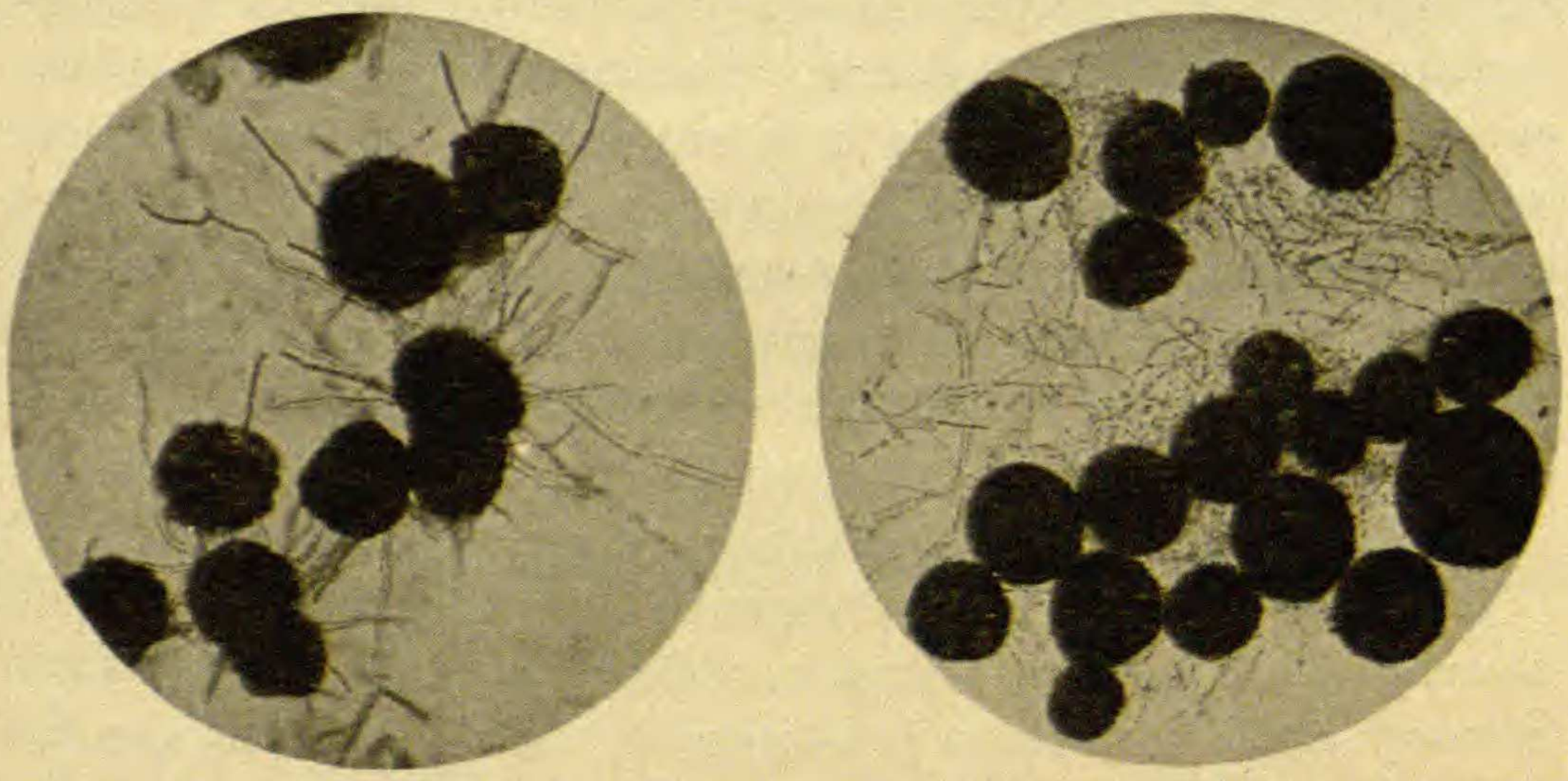
small, more or less inconspicuous, and sparingly developed. There are a number of basidiomycetous forms that produce bulbils as an imperfect condition. In a former contribution (6) the writer has referred to 4 such species, and in the present article 2 additional ones are described. The reddish orange color of the bulbil under consideration readily distinguished it from other species having clamp connections. The bulbils of *P. nigra* and *P. anomala* are dark brown or black, those of *Corticium alutaceum* chocolate brown, and those of *Grandinia crustosa* straw colored with conspicuous clamps.

Samples of the fruiting bodies of *Sporodesmium aurantiacum* B. and C., collected by Dr. THAXTER at Cranberry, North Carolina, in August 1889, were obtained from him for comparison with the bulbils of *P. aurantiaca*. As these structures were too old to germinate, a comparison of their mode of development could not be made. The fruiting bodies of the two fungi resemble each other so closely in their general form, color, texture, etc., however, that there is little doubt but that they are identical.

DEVELOPMENT OF BULBIL.—In common with many other bulbils, those of *P. aurantiaca* begin by a short lateral branch coiling up spirally. The early stages in the development, with some of the variations, are illustrated in figs. 25–38. During the process of coiling, which seldom results in more than two turns, the individual cells comprising the primordium become well supplied with food material and often appear distended (figs. 26, 27, 29). From the cells composing the coil short branches are developed (figs. 27, 28, 31, 32). These secondary branches may twine about each other or they may enlarge, forming cells that resemble those produced by a process of gemmation in other bulbils. These short branches and cells continue to be formed, sometimes on the concave side of the curve, sometimes on the convex side, until eventually all trace of the original coil has disappeared and the bulbil takes on the appearance of a homogeneous mass of cells, more or less irregular in shape, but on the whole somewhat spherical. Not infrequently around the margin of the developing bulbil numerous free twining ends of short branches are seen in loops and coils that are more or less characteristic (figs. 37, 38). This condition is also seen in

the fruiting bodies of *Sporodesmium aurantiacum* B. and C., already mentioned. In their early development the bulbils are usually very irregular in outline, owing to the projection of secondary branches which become less prominent in the mature form.

Frequently the bulbils appear as orange or yellowish patches scattered over the surface of the culture instead of being distributed evenly. This is due to the fact that the primordia are often produced in large numbers on a single branch, as shown in figs. 35, 36. As these develop, a corresponding number of bulbils are produced, which adhere together for a considerable time, superficially



FIGS. 5, 6.—*P. nigra:* fig. 5, group of germinating bulbils; fig. 6, group of mature bulbils, showing general form and variations in size.

resembling sclerotia. As a rule, these bulbils develop very slowly, usually taking several months before they mature. Eventually, however, as the substratum becomes dried up, the individuals separate into powdery, orange colored masses. The bulbils germinate readily in nutrient fluid, several of which are shown in text fig. 4.

**Papulospora nigra,** n.sp. (figs. 40–47; text figs. 5, 6).—Mycelium white, procumbent, scanty, oil globules and clamp connections conspicuous; bulbils colorless at first, becoming dark brown to black, nearly spherical, 100–180 $\mu$ in diameter at maturity; primordium one or more short lateral branches which coil up and intertwine. No other means of reproduction at present known.

On old cardboard, Cambridge, Massachusetts, and on hardwood chips, Seattle, Washington.

*Papulospora nigra* was obtained from gross cultures of old cardboard in the cryptogamic laboratories of Harvard University, and on similar cultures of chips in the botanical laboratory of the University of Washington, Seattle. When the bulbils appeared, pure cultures were made in a manner similar to that already described. This species has been grown on a variety of media for 8 years without the perfect condition being obtained. The mycelium is white and remains so throughout the period of rapid growth. Only when the hyphae get old do they begin to change color, becoming brownish or smoke colored. The primary mycelium is procumbent and on most media is inconspicuous, but becomes more or less flocculent or cobwebby on bran or prune agar. When a culture becomes old, the whole surface is covered with black bulbils which completely obliterate the mycelium. The hyphae frequently contain many large, conspicuous oil globules (figs. 40–42). The mycelium also has quite prominent clamp connections, a condition indicating its relation to the Basidiomycetes.

The bulbils of this species resemble closely those of *P. anomala* Hotson (6) in size, form, and color. They are readily distinguished, however, by their mode of development. In the latter species the bulbils arise from "slightly swollen, colorless, intercalary cells . . . . about 4 or 5 $\mu$ in diameter, sometimes projecting considerably and resembling short stunted branches; at other times the base of a short lateral hypha swells slightly and forms the primordium." From these primordial cells branches are sent out in different directions, the lateral walls of the basal cells adhering firmly together and becoming eventually incorporated into the bulbils. It will be seen that the development of the bulbil of *P. nigra* is quite different from this. It has already been shown in the consideration of *P. aurantiaca* that the bulbils of *P. nigra* may readily be distinguished from those of *Corticium alutaceum*, and also from those of *Grandinia crustosa* by their color.

DEVELOPMENT OF BULBIL.—From the primary hyphae short lateral branches, which coil up spirally, arise, producing one or two turns (figs. 42, 43). From the cells of these spirals short branches

are developed which intertwine, sometimes incorporating the primary filament. If the lateral branch divides, as it not infrequently does, the two filaments thus formed coil up, and these with those that are subsequently produced from them intertwine (figs. 44, 45). During the early stages of development the cell walls are usually clearly distinguished, but as the bulbil grows they become more or less transparent and quite indistinct (figs. 45, 46). At the stage represented in fig. 46 the whole bulbil is colorless, the cells containing a large number of oil globules, which condition continues until almost maturity, when they begin to turn brownish. The walls gradually become more pronounced, and on account of lateral pressure they assume a more definitely angular condition. As the bulbils increase in size they become more and more spherical, so that at maturity they have a clear cut, even margin. Text fig. 6 represents a group of these bulbils. Although they vary considerably in size, the general spherical form and even outline is maintained throughout. Sometimes elongated, irregular bulbils are formed when two primordia happen to be close together and fuse as they develop. These, however, are the exceptions, and the cause of their abnormal condition can usually be detected. If the bulbils are produced rather sparingly or away from each other, they invariably become spherical.

These bulbils germinate readily in sterile water in a Van Tieghem cell or in a watch glass. Fig. 47 illustrates the germination of a bulbil, 100 $\mu$ in diameter, after 48 hours in a hanging drop. Text fig. 5 represents germinating bulbils after 3 days. It may be noticed that they are not so even in outline as in text fig. 6. The probable reason for this is that, as the hyphae are produced, the marginal cells become forced aside and disarranged, particularly when the germinating tubes come from other than cortical cells.

**Papulospora magnifica,** n.sp. (figs. 39, 48–69).—Mycelium white, procumbent, scanty; bulbils light brown, becoming darker with age, spherical, 37–50 $\mu$ in diameter, with one, occasionally two large central cells surrounded by a single row of cortical ones which become empty at maturity; the primordium a short lateral branch of which the terminal and occasionally also penultimate cell enlarge.

On horse dung in moist chamber cultures, New York City.

In June 1915 the writer obtained a pure culture of *Papulospora magnifica* from Dr. B. O. DODGE for identification, with permission to make a cultural study of it. The fungus was originally found in New York City in April 1912, associated with *Ascobolus magnificus* Dodge, growing on horse dung in moist chamber cultures. DODGE (3) is inclined to consider this as parasitic on the mycelium of *A. magnificus*, having traced "a direct connection between the mycelium of the parasite . . . . and the mycelium of the host." He also shows by figures this definite connection. In a later statement (4) he suggests that the *Papulospora* may be associated with *Ascobolus magnificus* "either as a parasite or as an asexual spore form of the *Ascobolus*. If the former is the case, the mycelium of the parasite is intrahyphal; if the latter is true, then the phenomenon known as 'Durchwachsung' is extremely complicated in the mycelium of this *Ascobolus*."

As has already been indicated, bulbils must in all instances be regarded as representing imperfect conditions of the higher fungi; and, like the members of other more or less clearly defined form genera, may be associated with perfect conditions included in wholly unrelated genera of the Ascomycetes and Basidiomycetes. A bulbiferous condition has been found associated with the genus *Cubonia* (6) belonging to the same family as *Ascobolus*, so that it is not inconsistent with the general characteristics of the form genus *Papulospora* to consider the bulbils of *P. magnifica* as an imperfect condition of *Ascobolus magnificus*. All efforts, however, have failed to obtain the ascocarp from pure cultures of the bulbils, although repeated attempts have been made to do so by growing the fungus on a great variety of media which were exposed to different constant temperatures. Although the majority of the species of *Papulospora* are undoubtedly saprophytic, there are some reported as parasitic. *P. parasitica* (Karsten) Hotson was described by KARSTEN (7) as parasitic on beets, while COSTANTIN (2) described *P. dahliae* as connected with the roots of dahlias, but does not state definitely that it is parasitic, although that is the general impression one obtains from his article.

In the light of the general characteristics of the genus *Papulospora* and the fact that the hyphae of *P. magnifica* have been

definitely traced for some distance inside the filaments of *Ascobolus magnificus*, we are led to the conclusion that the fungus under consideration is parasitic on the latter rather than that the bulbil is the imperfect condition of it. On all the cultures made of *P. magnifica* the mycelium grew very sparingly, being procumbent, and at times growing down into the medium, but never becoming flocculent or aerial. On potato, bran, prune, and cornmeal agar only a small amount of mycelium was produced even after several months. So meager was the development that it might easily have been overlooked unless examined carefully with a hand lens. Of the different media tried, a decoction of horse dung with agar or the horse dung itself, sterilized in an Arnold's steam sterilizer, proved the most satisfactory.

A microscopic examination frequently showed the mycelium to be a network of anastomosing hyphae (fig. 69), while at other times (figs. 65–68) enlarged food storage cells were found, the largest being 15 $\mu$ in diameter.

DEVELOPMENT OF BULBIL.—The primordium of the bulbil is quite easily recognized as a short lateral branch, somewhat coiled or curved and well filled with granular material. In this development the bulbil seldom, if ever, produces a coil crosier fashion, such as does *P. parasitica*, which it most closely resembles. From the end of this coiled branch a cell is cut off, enlarges, and becomes well filled with granular food material (figs. 54, 55). This cell eventually develops into the large central cell of the bulbil. Occasionally this lateral branch twists on itself, as represented in figs. 51, 53, while at other times a secondary branch is formed from it (figs. 50, 52). The usual mode of procedure, however, is that shown in figs. 48, 49, 54, 55. It may be seen that the end cell continues to enlarge, subsequently becoming almost spherical, reaching a diameter of 10–20 $\mu$. Before it reaches its mature size, however, several short branches, which grow over the surface, are given off from it (figs. 55–59). These branches intertwine, clinging close to the wall of the enlarged cell, finally inclosing it, so that the mature bulbil consists of a single large central cell, rich in food material, surrounded by a layer of cortical cells produced by these branches becoming compacted firmly together

laterally. In the course of development these outer cells lose their protoplasmic contents, although the walls retain more or less of the brownish color.

Although the foregoing description of the mode of development of the bulbil is the usual one, not infrequently a second large cell is formed by the primordial branch (figs. 62, 63). In such instances the further development is practically the same as where there is a single central cell. The lateral branches which eventually become the cortex are produced from both the large cells, which subsequently become completely surrounded, precisely as in the case already described.

### Germination of bulbil

The bulbils of most of the species of *Papulospora* germinate with little difficulty. All of those described in this article, with the exception of *P. magnifica*, have been found to produce germ tubes quite readily. In the study of that species various media were employed in the hope that a favorable condition might be found for the germination of the bulbils. Among these were bran, potato, and prune agar, various synthetic media, as well as decoctions of horse dung used both as a liquid and associated with agar, but all these failed to produce the desired result. Finally a method that the writer had found successful in inducing the ascospores of certain species of *Ascobolus* and *Cubonia* (6) to germinate was tried with some success. Mature bulbils were put on a flamed glass slide and carefully crushed with the flat surface of a scalpel. They were then transferred to hanging drops of nutrient media, a sterile decoction of horse dung proving the best. Many of the bulbils thus crushed were totally destroyed, but in a few instances, where the pressure was just sufficient to break the cortical layer of cells without injuring the large central one, germination was produced and a branching filament soon developed (fig. 39).

The mature bulbil of *P. magnifica*, with one or two large central cells surrounded by empty cortical ones, superficially resembles certain bulbils of *P. coprophila* (Zukal) Hotson. The latter, however, consists of 1–4 (sometimes as many as 10) large central cells, only occasionally having a single central cell. Moreover, the

spiral primordium of *P. coprophila* and the flocculent and abundant mycelium differ widely from those of *P. magnifica*. The bulbils of *P. magnifica* more closely resemble those of *P. parasitica* (Karsten) Hotson than they do those of *P. coprophila*. However, in *P. parasitica*, which is described as parasitic on beets in the original description by KARSTEN, the mycelium is flocculent, the bulbils 15–21 $\mu$ in diameter, with a single large central cell invariably present, and the primordium a spiral which coils crosier fashion. Thus, the procumbent and scanty character of the mycelium of *P. magnifica*, as well as the size and mode of development of the bulbil, readily distinguish it from *P. parasitica*. In order to obtain further information regarding the relationship of these two fungi, inoculations were made in the roots of growing beets and turnips, both in the field and in the laboratory. In each case a small slice of the root was removed with a sterile knife and a cavity made in the cut surface. From a pure culture of *P. magnifica* a portion of the nutrient agar containing bulbils and mycelium was gouged out and deposited in this cavity. Over this a small piece of glass was put and the soil replaced. Although several similar experiments were carried on, no indication of a parasitic condition could be detected.

Other species that resemble the two just mentioned, such as *Physomyces heterosporus* (*Monascus heterosporus* [Harx] Schröter), *Dendryphium bulbilferum* Zukal, *Acrospeira mirabilis* Berk. and Br., etc., have already been discussed (**6**), so that it is not necessary to repeat the discussion.

## Key to species of bulbiferous fungi

There are several more or less well defined characteristics that are made use of in making the following key for the members of the form genus *Papulospora*. A broad division is readily made on the presence or absence of clamp connections in the mycelium. Those forms which do not have this condition are grouped into 4 categories based on the color and size of the bulbil, namely, colorless to cream, steel gray, black, and yellowish red to dark brown. Another character used, especially in the last mentioned division, is the mode of development of the bulbils, whether from intercalary

cells, a single lateral branch, or a group of vertical hyphae. Using these characters as a fundamental basis for separation, the described species of bulbiferous fungi may be distinguished as follows:

Hyphae with clamp connections
  Bulbils dark brown to black
    Bulbils 65–80 $\mu$ in diameter, chocolate brown . . . . . *Corticium alutaceum*
    Bulbils 125–175 $\mu$ in diameter, dark brown or black; margin even
      Primordium intercalary . . . . . *Papulospora anomala*
      Primordium spiral . . . . . *Papulospora nigra*
  Bulbils light yellow, 52–88 $\mu$ in diameter; hyphae conspicuous, ropelike *Grandinia crustosa*
  Bulbils yellow, becoming orange, 100–250 $\mu$ in diameter; hyphae formed evenly . . . . . *Papulospora aurantiaca*
Hyphae without clamp connections
  Bulbils colorless, pale yellow, or cream colored
    Bulbils cream colored, 30–35 $\mu$ in diameter; parasitic on *Geoglossum* *Papulospora candida*
    Bulbils colorless or pale yellow, 70–100 $\mu$ in diameter, saprophytic *Papulospora pallidula*
  Bulbils steel gray, 21–36 $\mu$ in diameter . . . . . *Papulospora cinerea*
  Bulbils black or smoke color
    Bulbils 75–100 $\mu$ in diameter; margin even . . . . . *Cubonia bulbifera*
    Bulbils 200–300 $\mu$ in diameter; margin irregular . . . . *Papulospora pannosa*
  Bulbils yellowish red to dark brown
    Bulbils scanty; perithecia usually present
      Perithecia with neck and lateral and terminal setae *Melanospora cervicula*
      Perithecia with papilla and terminal setae . . . . . *Melanospora papillata*
    Bulbils abundant; perithecia usually absent
      Primordium intercalary
        Bulbils brownish yellow; central cells 28–55 $\mu$ in diameter *Papulospora immersa*
        Bulbils straw color; central cells 10–20 $\mu$ in diameter *Papulospora irregularis*
      Primordium one or more lateral branches
        Primordium normally a single lateral branch
          Primordium spiral
            Cells of the bulbil heterogeneous; cortex definite
              Normally only one central cell
                Cortex complete
                  Mycelium scanty, procumbent; bulbil 37–50 $\mu$ in diameter; occasionally two central cells *Papulospora magnifica*

Mycelium abundant, flocculent; bulbil 15–21 μ in diameter; invariably 1-celled. . . *Papulospora parasitica*
Cortex incomplete. . . . . *Acrospeira mirabilis*
Normally more than one central cell
Spiral in one plane; cortical cells spinulose
*Papulospora spinulosa*
Spiral normally in more than one plane; not spinulose; 2–6 central cells
Bulbils dark brown. . . . . *Papulospora coprophila*
Bulbils brick red. . . . . *Papulospora rubida*
Cells of bulbil homogeneous throughout
Bulbils chocolate brown, 21–36 μ in diameter, producing *Sporotrichum* spores. . . . . *Papulospora sporotrichoides*
Bulbils straw color, 100–250 μ. . . . . *Papulospora byssina*
Primordium not spiral; bulbils large, irregular, 100–750 μ in diameter. . . . . *Papulospora aspergilliformis*
Primordium two or more lateral branches forming a spherical aggregation of cells at the top. . . . . *Papulospora polyspora*

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## EXPLANATION OF PLATES XXI–XXIII

The drawings for the plates were made with the aid of a camera lucida, using different combinations of the Bausch & Lomb lenses. All the stages

in the development of bulbils were drawn with the same magnification, using 4 mm. objective and no. 12 eyepiece. The text figures are microphotographs taken by W. J. WESTERBERG. The plates have all been reduced in reproduction about three-fourths.

FIGS. 1–16.—*Papulospora pallidula.*

FIG. 1.—Hypha showing large oil globules.

FIGS. 2–6.—Dichotomously dividing primordium.

FIGS. 7, 8.—Primordia more or less irregular in their dichotomous branching.

FIG. 9.—Further development of bulbil.

FIGS. 10–12.—Second mode of forming a bulbil.

FIG. 13.—Mature bulbil germinating.

FIG. 14.—Cells of an old bulbil loosely adhering to each other; some of cells germinating.

FIGS. 15, 16.—Terminal primordia.

FIGS. 17–24.—*Papulospora byssina.*

FIGS. 17–19.—Different forms primordium may assume.

FIGS. 20–22.—Stages in development of bulbil.

FIG. 23.—Germinating bulbil.

FIG. 24.—Primordia of at least 3 bulbils from same filament at *a*, *b*, and *c* respectively.

FIGS. 25–38.—*Papulospora aurantiaca.*

FIGS. 25–32.—Variations in mode of coiling of primordium of bulbil.

FIGS. 33–38.—Other states in development of bulbil.

FIG. 39.—Germinating bulbil of *Papulospora magnificus.*

FIGS. 40–47.—*Papulospora nigra.*

FIG. 40.—Portion of hypha showing large oil globules and clamp connection.

FIG. 41.—Form of primordium that sometimes occurs.

FIGS. 42–46.—Successive stages in development of bulbil.

FIG. 47.—Mature bulbil germinating.

FIGS. 48–69.—*Papulospora magnifica.*

FIGS. 48, 54–60.—Stages in development of bulbil.

FIGS. 49–53.—Forms of primordium that occasionally appear.

FIG. 61.—Median section of mature bulbil.

FIGS. 62, 63.—Stages in development of bulbil with 2 large central cells.

FIG. 64.—Median section of mature bulbil with 2 large central cells.

FIGS. 65–68.—Portions of hyphae with large storage cells densely filled with food material.

FIG. 69.—Portion of normal mycelium showing tendency to anastomose.